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ORIGINAL ARTICLE

The influence of altered functional loading and posterior bite-blocks on the periodontal ligament space and alveolar bone thickness in rats

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ABSTRACT

Objectives: Posterior bite-blocks are resin-based structures elevating the occlusion and creating intrusive force on the posterior teeth. Bite-blocks were applied to the molars of growing rats and a hard and soft diet was used to create altered functional masticatory forces. The aim of the present investigation was to study the effect of this appliance on the periodontal ligament space and alveolar bone thickness when combined with altered masticatory forces.

Material and methods: Fifty-two four-week-old rats were divided into two groups, hard and soft diet. Two weeks later, half of them received a bite-block appliance, creating four groups: control hard (CH), control soft (CS), bite-block hard (BH) and bite-block soft (BS). All were sacrificed at age of 10 weeks. Their heads were scanned by micro-CT and periodontal ligament space (PDL) width, cross-sectional alveolar socket surface and alveolar bone thickness were measured. Analysis of variance (ANOVA) was used to compare the groups.

Results: The PDL was 9.2% thinner in the CS group ($p < 0.001$) and 20.7% in the bite-block groups ($p < 0.001$) compared to the CH group. Within each of the four groups, the mesiodistal PDL space was larger than the palatobuccal. The alveolar bone was thinner by 5.8% ($p = 0.018$) in the CS group, 10.7% in the BH group ($p < 0.001$) and 16.7% in the BS group ($p < 0.001$) compared to the CH group.

Conclusions: Young rats wearing posterior bite-blocks have narrower PDL space and thinner alveolar bone compared to controls. When fed a soft diet, the alveolar bone is even thinner but the PDL showed no difference.

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Introduction

Posterior bite-blocks are appliances used in orthodontic treatment of anterior open-bites [1–3] in growing patients. These resin-based structures are placed between the teeth and elevate the occlusion vertically. This produces an intruding force [2] on the posterior teeth through the stretched masticatory muscles and soft tissues, which leads to autorotation of the mandible and closure of the anterior open bite. In addition to the dental response, this appliance has a wide range of effects. Clinical studies have shown that it decreases the lower face height [4,5] and decreases the electromyographic (EMG) activity of masticatory muscles [6], more specifically the masseter and temporal muscles. Furthermore, a more acute gonial angle [5] and an upward and forward growth [4,5] of the mandible, produced by the intrusive effect on the posterior teeth, were also observed and contributed to the decrease in lower face height.

Experimentation on animals has demonstrated similar findings. In monkeys, posterior bite-blocks produced changes in mandibular size and condylar form [7], as well as changes in the dental position.[8] In the rat, the mandible undergoes a forward rotation and results in a decrease in the total viscerocranium height.[9] Muscle activity is reduced [10], the masseter muscle stretches [11] and the molars have been shown to undergo intrusion.[9] Studies have also shown modifications in the alveolar bone quality, including its cortical density, height [12,13] and number of trabeculae.[14]

It has been previously shown that there is a wide range of variation between individuals' functional capacity as it is expressed in maximal bite-force.[15–17] Thus, these interindividual differences in bite-force can potentially affect tissue response to bite-blocks, by adding a dynamic component to the static effect of the appliance on the surrounding tissues. By elevating the occlusion, bite-blocks apply a constant static intrusive force on the teeth, but in addition, they might also interfere with the dynamic forces brought on by mastication. Thus far, in animal studies, the absence or decrease of functional forces was shown to reduce the periodontal ligament space [18–23], indicating the role of this tissue as a shock absorber of occlusal forces. In the current study, our goal was to examine the effect of bite-blocks on the periodontal ligament space and alveolar bone thickness, in the context of variable masticatory forces created with a soft and hard diet, by placing them on the molars of young rats.

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Materials and methods

Animals

Fifty-two Sprague–Dawley 3 weeks old male rats were quarantined one week. At 4 weeks of age, they were separated into two equal groups, hard and soft diet to produce different functional capacities and at 6 weeks of age each group was again divided into two equals groups, bite-block and control. The soft diet was achieved by mixing water with ground food pellets, creating a soft paste. The bite-blocks were made from composite resin and measured 3×7 mm, covering entirely the three molars. They elevated the occlusion by 2 mm at the first molar and 1 mm at the third molar, to follow the geometric opening of the mandible. They were glued to the upper teeth on both sides with Vitrebond cement (3M Dental Products Division, St Paul, MN). We used the same specimen as this previous paper.[9] At 10 weeks of age, the four groups, control hard diet (CH), control soft diet (CS), bite-block hard diet (BH) and bite-block soft diet (BS) were sacrificed and their heads preserved in a solution of 99% ethanol.

Micro-CT scanning

We used the Skyscan 1076 micro-CT (Buker-microCT, Kontich, Belgium) for digitizing the skulls. All scanning was performed with identical parameters: 140 mA, 70 kv, 2945 ms exposure, Al 1.0-mm filter, 0.2 rotation step, 180° scan and nine-micron nominal resolution.

Reconstruction

Using NRecon reconstruction software (Buker-microCT, Kontich, Belgium), we reconstructed each maxillary molar region separately, thus creating separate files for left and right, with the following parameters: ring artefact correction: 6, beam-hardening correction: 20%, dynamic range (contrast and brightness): 0.0052–0.1454. The reconstructed images were then re-sliced with Osirix (Geneva, Switzerland) using the same parameters as in a previous study.[24] The image series were randomized to hide the group and side information from the operator.

PDL space, cross-sectional alveolar socket surface (as)

The data analysis was also done with Osirix. The measurements were done on the roots of the first (roots 3–5) and second (roots 6–9) molars and the orientation was based on the axes of roots 8–9 (palatobuccal) and roots 5–7 (mesiodistal) (Figure 1). Roots 1 and 2 were excluded from the study, because they are very different in shape and size in comparison to the other roots. The polygon tool was used to measure the area and circumference of the alveolar socket and the root at three different levels: cervical, midroot and apical (Figure 2). The apical and cervical levels were taken at 270 microns from the apex and furcation, respectively, and the mid-root was the calculated halfway section. From these data, we calculated the PDL width. In addition, we also measured the PDL width with the line tool separately at four different regions: mesial, distal, palatal and buccal.

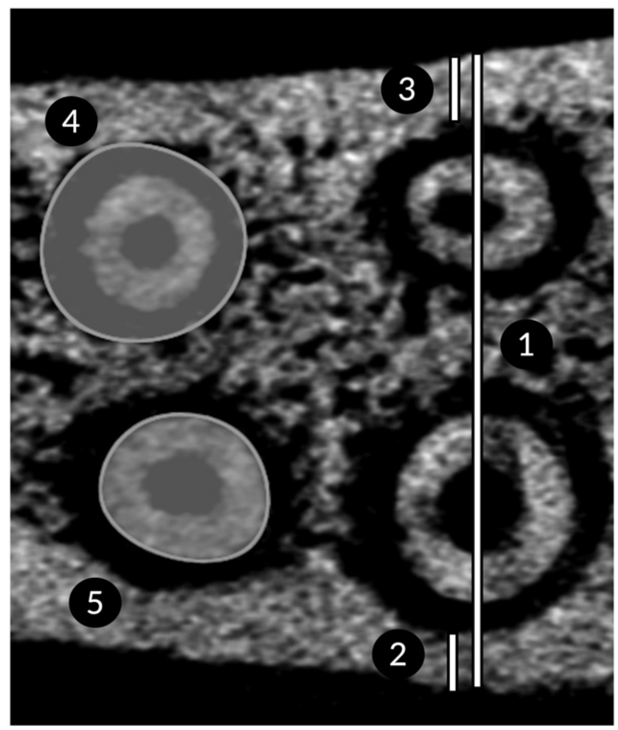

Figure 2. The measurements performed with Osirix. (1) alveolar process width, (2) palatal bone width, (3) buccal bone width, area and perimeter measurements of the (4) alveolar socket and (5) root.

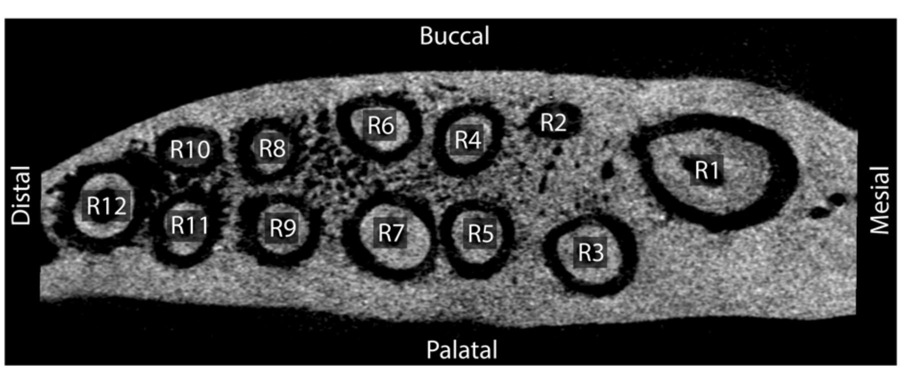

Figure 1. Horizontal section of the maxillary alveolar process passing through the roots of the three maxillary molars. Roots 1–5 belong to the first molar, 6–9 to the second and 10–12 to the third.

These measurements were then grouped into mesiodistal and palatobuccal regions. The measured alveolar socket surface was also used.

Alveolar process width

Measurements were taken at the root pairs 4–5 and 8–9 at the average midroot level. We measured the total width of the alveolar bone as well as the palatal and buccal bone widths at the average mid-root level of the distal roots, of the first and second molars (Figure 2). These two regions were averaged for analysis.

Statistical analysis

SPSS Statistics (IBM Corp., Armonk, NY) was used to compare the four groups or the levels within each group. We performed a one-way ANOVA with a level of significance set at $p < 0.05$ and a Tukey's HSD *post hoc* test. Analysis of mesiodistal with palatobuccal PDL space within each group was performed with a paired *t*-test. The variables side and root, as well as the region for the alveolar process width measurement, were averaged for analysis.

Method error

Thirty measurements of each variable were performed twice and analysed with Dahlberg's formula to access the random error and a paired *t*-test to calculate the systematic error. The random error measured was 6.8 (μm) for the PDL, 14.3 (10^3 μm^2) for the alveolar socket surface and 54.8 (μm) for the total alveolar width. The paired *t*-test showed no significant differences for systematic error. The coefficient of reliability was calculated following Houston's method [25] and resulted in 99.2% for the PDL, 87.8% for the alveolar socket surface and 97.9% for the alveolar bone width.

Results

Periodontal ligament space

The periodontal ligament space was smaller on average in the control soft group compared to the control hard group at every level ($p < 0.001$). On the contrary, there was no difference between the groups with bite-blocks either with soft or hard diet in any level. On average and at the cervical and midroot levels, the use of the bite-block, whether with hard or soft diet, led to a smaller PDL compared to controls ($p < 0.001$). However, at the apical level, the bite-block groups were only slightly smaller than the control hard group ($p < 0.001$) and there was no observed difference with the control soft group. In the control soft group, the size of the cervical PDL was bigger than at the apical level ($p = 0.049$). In the bite-block groups, the apical level was much larger than the cervical and mid-root levels ($p < 0.001$) (Figure 3). In general, the average mesiodistal PDL thickness was larger than palatobuccal one in all groups ($p < 0.001$). However, the difference was twice the size in the control soft diet group compared to the control hard group (Table 1). Between the groups, the PDL thickness in the mesiodistal and palatobuccal regions was smaller in the control soft group compared to the control hard group ($p < 0.001$) and even smaller in the bite-block hard and soft groups ($p < 0.001$) (Figure 4).

Alveolar socket surface

In the groups without bite-block, the alveolar socket surface was smaller at all levels in animals fed a soft diet ($p < 0.01$) and similarly to the measurements of the PDL; in the bite-

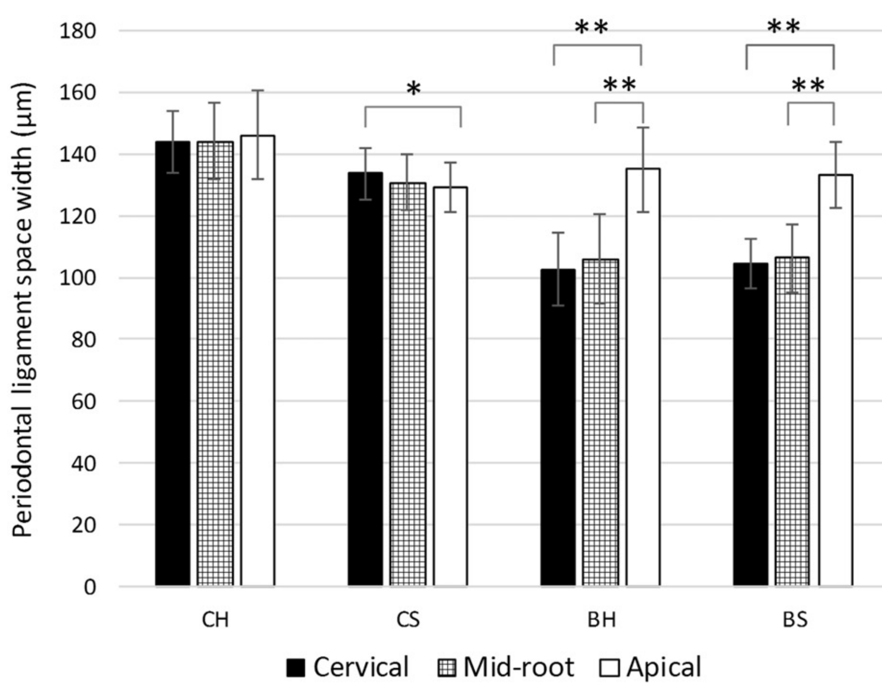

Figure 3. Comparison of the periodontal ligament space width by group and level. Error bars represent the standard deviation. *$p < 0.05$; **$p < 0.001$.

Table 1. Comparison of mesio-distal and palato-buccal PDL space within each of the four groups.

	Mesiodistal		Palatobuccal		
PDL space (μm)	Mean	SD	Mean	SD	*p* value
Control hard	142.2	22.0	134.2	23.8	0.000
Control soft	135.6	22.6	117.5	25.1	0.000
Bite-block hard	112.5	30.2	107.7	30.9	0.000
Bite-block soft	115.8	30.6	109.3	32.6	0.000

Means and standard deviations given for each group.

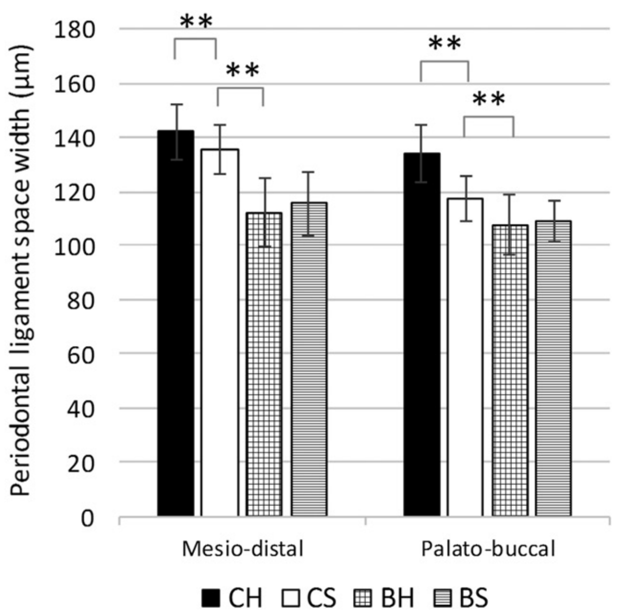

Figure 4. Four experimental groups compared for periodontal ligament space width in the mesiodistal and palatobuccal regions. The three levels were averaged. Error bars represent the standard deviation. *$p < 0.05$; **$p < 0.001$.

block groups, there was no difference between hard and soft diet. The alveolar socket surface in the bite-block groups were smaller on all levels compared to the groups without bite-blocks ($p < 0.009$) except for the apical level between the control soft and bite-block hard groups, where no difference was shown. The control hard and soft groups had progressively smaller alveolar socket surface towards the apex ($p < 0.001$), but the bite-block groups show little difference: the bite-block hard group was smaller only between the cervical and midroot levels ($p = 0.011$) and the bite-block soft group between the cervical and midroot ($p < 0.001$) and apical levels ($p < 0.001$), respectively. (Figure 5).

Alveolar bone width

The total width of the alveolar bone was gradually smaller between the groups: the control hard group was the widest, followed by the control soft group, then the bite-block hard and finally the bite-block soft group ($p < 0.05$). On the palatal side, the control hard group had the thickest bone ($p = 0.042$), the bite-block soft the thinnest ($p < 0.001$) and there was no difference between the control soft and bite-block hard groups. On the buccal side, the control hard group was larger than the other groups ($p = 0.017$), but there was no difference between control soft, bite-block hard and bite-block soft groups. (Figure 6).

Discussion

The present study has shown that animals with bite-blocks had a smaller PDL space mainly of the cervical and midroot regions compared to the control groups and that different functional demands had no effect on the PDL thickness when the bite-block was used. The alveolar bone was also smaller in the rats wearing the appliance compared to controls and in-between bite-block groups it was smaller in animals fed a soft diet. The difference in alveolar bone width observed between bite-block hard and soft groups suggests that the bite-blocks' effect on the alveolar process can vary between individuals of different functional capacity.

The use of bite-blocks reduces the PDL space more extensively than the effects of the low functional demands of a soft diet compared to animals fed the 'normal' hard diet. The PDL space is smaller [18–23] in animals with decreased functional demands compared to the controls due to the reduction in masticatory occlusal forces. The fact that the use of bite-blocks decreases further this space may be linked to the combined effect of decreased masticatory forces due to reduced muscle activity [10] and reduction in independent tooth mobility, due to the splinting effect of the appliance. It is also possible that the flat occlusal plane of the bite-blocks reduces forces through variation of the angle of application of masticatory forces; however, Katona's [26] findings remain mainly theoretical.

At the apical level in the bite-block groups, the situation seems different than the rest of PDL. In the control groups, the difference between the cervical, midroot and apical levels is very small. However, the apical level of the bite-block groups is larger than the cervical and mid-root levels due to the size of the alveolar socket surface. In the control groups, the alveolar socket surface becomes smaller towards the apex, as it follows the natural anatomy of the root. In the bite-block groups, there is no difference between the apical and midroot levels, which accounts for the enlarged PDL thickness of the apical level. It is possible that the intrusive occlusal pressure from the bite-blocks creates a pressure zone that resorbs the neighbouring bone and enlarges the alveolar socket surface and PDL at the apical region.[27] After the initial period of resorption, as no tooth movement occurs, the bone position would stabilize and reform. This is consistent with a previous study, where it was observed that the bone directly underneath the apex in rats with bite-blocks had increased bone mineral density compared to controls.[28] Elongated crown height of teeth with bite-blocks may also be a contributing factor by increasing the moment of the masticatory forces (lever effect) and creating more movement at the apex, although due to the flat occlusal plane (from the bite-block) the horizontal components of the occlusal force may be limited. They depend on the friction between the mandibular teeth and the bite-block and the arch trajectory during chewing.

Within each of the four groups, the mesiodistal PDL is consistently smaller than the palatobuccal PDL space. In the bite-block groups, the palatobuccal and mesiodistal regions were equally affected compared to the control hard group. However, the animals with reduced functional demands due

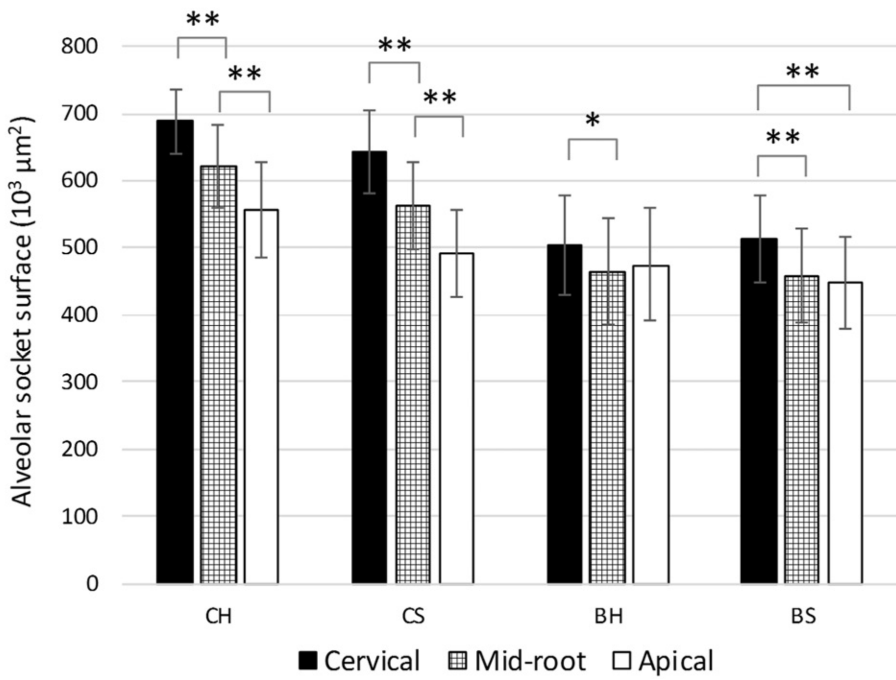

Figure 5. The cross-sectional alveolar socket surface, comparison between the four groups and three levels. Error bars represent the standard deviation. $*p < 0.05$; $**p < 0.001$.

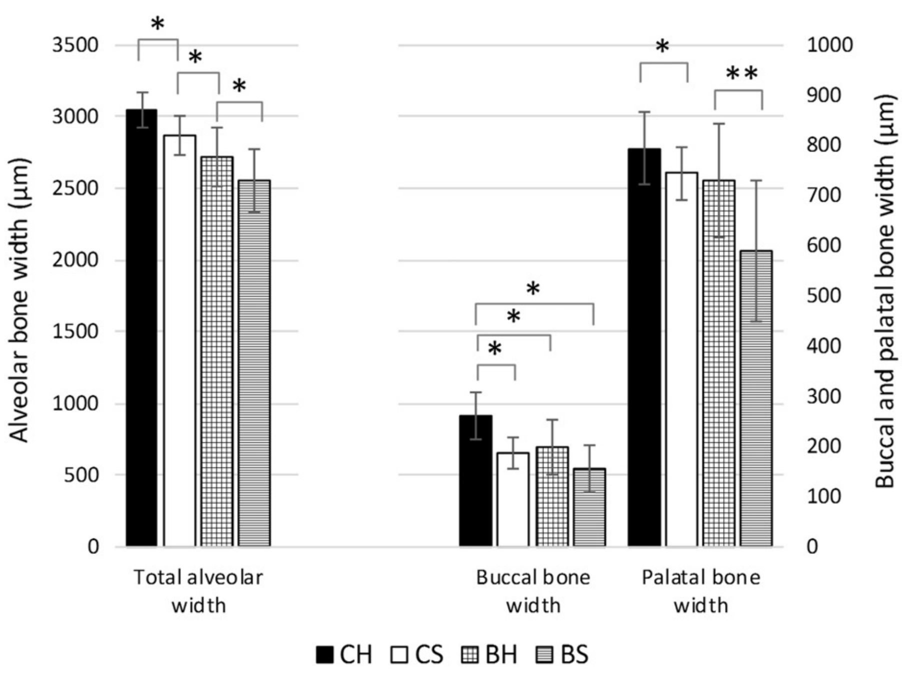

Figure 6. Alveolar bone measurements. The total alveolar process width and the buccal and palatal bones width. Comparison between the groups. Error bars represent the standard deviation. $*p < 0.05$; $**p < 0.001$.

to the soft diet showed twice as much reduction in the palatobuccal than the mesiodistal regions and the difference between the two regions was two times larger than the control group. This may be explained by the physiological mastication cycle: masticatory forces act on the tooth from a variety of angles due to the transverse and horizontal components of a normal mastication cycle.[29] Our results suggest that masticatory forces while chewing a hard diet have a more important stimulatory action in the palatobuccal direction compared to the mesiodistal direction, even though masticatory forces act in both axes. The greater reduction in size of the PDL in the control soft group suggests that the palatobuccal action is reduced when feeding animals a soft diet.

In contrast to the animals fed a soft diet, the bite-block groups were equally affected in both directions, which indicates that in addition to the reduced occlusal forces, they may be exposed to reduced mesiodistal mobility. The appliance is attached by gluing and in effect, splints the teeth together. Immobilizing the teeth may reduce the mesiodistal and the palatobuccal forces that are produced, which could explain that the PDL in the bite-block groups is equally reduced in both regions.

Decrease in PDL thickness is accompanied by atrophic changes, such as fewer and smaller blood vessels [21,30], fewer fibroblasts [31], disorganized collagen fibres [19,20,23,31–33] and decrease in periostin expression.[34,35] This state may reduce the ability of the PDL to heal and modify treatment outcome in the case of dental trauma. This is consistent with the literature that promotes splinting only in the initial weeks following trauma after which it is to be removed in order to allow occlusal stimulation.[36,37]

The alveolar bone receives functional stimulation from the teeth by the mediation of the PDL. Like any other bone in the human body, it adapts to its physical needs and as a result the alveolar process width decreases with soft diet and bite-block treatments.

Comparing to control animals, the alveolar bone width is narrower in descending order, in rats fed a soft diet, in animals with bite-block and hard diet, and in those with bite-block and soft diet. This shows that either masticatory forces continue to affect bone stimulation in rats while wearing the bite-blocks or that this difference is a left over effect of occlusal stimulation (hard vs. soft diet) during the first two weeks of experiment, before the introduction of the appliance. Apart the functional stimulation of the bone through mastication, bite-blocks elongate the tendons of masticatory muscles, which may cause a constant vertical pressure. This constant pressure possibly does not influence the alveolar bone width because it has been shown that bone is not stimulated by continuous forces.[38]

In conclusion, the bite-blocks affect the alveolar process in several ways. Rats wearing the appliance had a thinner periodontal ligament and narrower alveolar bone compared to the control group. When the appliance is combined with a soft diet instead of the 'normal' hard diet, there is no difference in the PDL space width but the alveolar bone is thinner.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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